Effects of clear-cutting on the composition of bacterial populations of northern spruce forest soil

SEPPO NIEMELÄ
University of Helsinki, Department of Microbiology, SF-00710 Helsinki 71, Finland
AND
VERONICA SUNDMAN
University of Helsinki, Department of General Microbiology, SF-00200 Helsinki 20, Finland

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This paper concerns the microbiological part of an investigation, the goal of which is to describe the biological changes in coniferous forest soil upon clear-cutting in a northern (66°20′ N) moraine area where reforestation after clear-cutting had been met with difficulty. The zoological part of the work has been published elsewhere. Clear-cut sites of increasing age (4, 7, and 13 years) were investigated and compared with a forest area where no cutting of timber had been done for 120 years.

A total of 684 random isolates of heterotrophic bacteria from pooled samples of the sites investigated were passed through 36 biochemical tests. The data were condensed by the aid of factor analysis, and a comparison of the populations was based on squared Euclidean distances between population centroids in a seven-dimensional factor space.

The most marked population changes followed a course in which frequencies of some population characteristics became increasingly different until 7 years after clear-cutting, with regression towards the control clearly evident after 13 years. Disturbances of shorter duration were also relatively common, with maximal changes observed in the 4-year samples, and with a complete recovery after 7 years.

The mineral soil populations seemed to undergo greater changes than the humus populations.

The most distinct changes believed to be due to clear-cutting were the short-term relative increase of organisms producing acid from sucrose and dissolving $CaHPO_4$, and a long-term increase of lipolytic and caseolytic, rhamnose-negative organisms; both in the mineral soil layer. In the humus layer, a short-term increase of lipolytic and of rhamnose-positive organisms seemed to take place.

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La présente publication porte sur la partie microbiologique d'une recherche dont le but était de décrire les changements biologiques qui surviennent dans un sol forestier de conifères, après abattage complet, sur une surface de moraine nordique (66°20′ N), là où la reforestation s'est avérée difficile après l'abattage. La partie zoologique de ce travail fut publiée ailleurs. Des sites mis à nu, d'âge croissant (4, 7 et 13 ans), furent comparés avec une région forestière où aucun abattage n'a eu lieu durant 120 ans.

Un total de 684 échantillons de bactéries hétérotrophes furent pris au hasard, à partir d'un assemblage d'échantillons provenant des sites d'investigation, et soumis à 36 essais biochimiques. Les données furent condensées à l'aide d'un facteur d'analyse, et une comparaison des populations fut établie par le carré des distances d'Euclide entre les populations centroï dans un espace à sept facteur dimensionnels.

Les changements de population les plus marqués ont suivi un cours dans lequel les fréquences de certaines populations caractéristiques devinrent de plus en plus différentes au cours de 7 années après l'abattage, avec une régression clairement évidente vers le témoin après 13 ans. Des perturbations de plus courte durée furent également relativement communes, avec un maximum de changement dans les échantillons de 4 ans, mais avec recouvrement complet après 7 ans.

Les populations du sol minéral ont semblé subir des changements plus marqués que celles de l'humus.

Les changements les plus notoires qui semblent causés par l'abattage sont, à court terme, l'augmentation relative d'organismes qui produisent de l'acide à partir du sucrose et dissolvent le $CaHPO_4$ et, à long terme, l'augmentation des organismes rhamnose-négatifs, lipolytiques et caséolytiques; ces changements prennent place dans la couche minérale du sol. Dans la couche humique, l'augmentation d'organismes rhamnose-positifs et lipolytiques semble prêndre place à court terme.

[Traduit par le journal]

Introduction

The need for thorough investigation at all biological levels has been demonstrated by failure of reforestation after clear-cutting of coniferous forests in northern Finland (e.g. Etholen 1972). After clear-cut sites in various regions were screened, an area was selected in Kuusamo where clear-cut sites of various ages were situated close to each other on the same geological formation. All sites originally had the same forest type. Practical difficulties limited the numbers of replications to a minimum. Two sampling sites of the same age were selected on a basis of superficial dissimilarity (dry and moist) to represent the maximal expected variation within sites of the same age after clear-cutting. Differences between these two were used as rough measures of stochastic variation within populations.

The qualitative comparison of the soil populations of the different sites to be described in this paper was founded on random samples of pure cultures taken on a single day. Confidence in such limited data is based on a three-way analysis of variance of plate counts of monthly samples during the unfrozen period. Since the relative differences in colony densities between the sampling sites remained unchanged throughout the summer, it was assumed that qualitative differences would also prevail, and any single day would be suitable for demonstrating the possible existence of such differences.

After the random isolates were checked for purity, they were passed through a set of physiological tests. Without any effort to identify individual isolates, the populations, i.e. groups of isolates which originated in one forest site, were described in terms of the pooled test data for that group by the aid of factor analysis (Cattell 1965; Hopkins 1967; Quadling 1967, 1970; Sundman 1970).

Monthly samples from the same sites during two successive growth seasons have been included in a study of the effects of clear-cutting on the invertebrate fauna (Huhta 1976).

Materials and Methods

Study Area and Sampling Sites

The study area was situated in the northern part of Kuusamo commune, in the immediate vicinity of Oulanka National Park (coordinates about 66°20′ N, 29°20′ E, altitude 260–270 m). The sampling sites represent the dominant forest type in the area, viz. a spruce stand nearest to Hylocomium–Myrtillus type (HMT) according to Kalela (1961). The soil type is a moraine with a podsol profile and an uppermost layer of 4–5 cm of raw humus. The pH of the different strata of the sampling sites varied between 3.5 and 5.0 as measured in distilled water. The mean temperature of the unfrozen period June–September in the year of sampling (1971) varied in the humus layers of the sampling sites between 6.8 °C (site 0) and 9.5 °C (site 4). The individual sites, named after the years passed since clear-cutting, were as follows.

Site 0—Control site (0 years from clear-cutting). An old (120 years) spruce stand mixed with pine and some birch (crown coverage about 30%), at the eastern border of the Oulanka National Park (Ampumavaara).

Site 4—(Four years from clear-cutting). Cut in winter 1967–1968. In the immediate vicinity of site 0. Area about 4 ha. Stocking with pine seedlings in 1968 failed. Shrub stratum missing.

Site 7—(Seven years from clear-cutting). An amount of 0.5 km from site 4, clear-cut in winter 1964–1965. Stocked with pine, but most of the saplings had failed to grow. Area about 6 ha. Spruce, birch, and mountain ash very sparsely in the shrub stratum.

Site 13a—(Thirteen years from clear-cutting). An extensive clear-cut area (total area about 6 km^2) west of the National Park (Ruoppiharju). Trees in the study site were felled in 1958. Restocking performed twice, but with little success; a few saplings, only about 20 cm in height, covered less than 2%. Sprouts of deciduous trees, mainly aspen, raised the total coverage close to 10%.

Site 13b—(Thirteen years from clear-cutting). A moist site in the same clear-cut area as site 13*a*. *Vaccinium uliginosum* and a dense carpet of *Polytrichum commune* reveal the process of embogging. *Betula pubescens* dominating in the shrub stratum. Otherwise as site 13*a*.

Exact analysis of the field and ground vegetation was presented by Huhta (1976).

Sampling and Sample Treatment

All samples for this qualitative study were collected on July 20, 1971.

At every sampling site five spots were selected close to each other, and at each spot a sample of the humus layer, 25 × 25 cm in area, was removed. The cakes of humus were put into individual plastic bags.

After removal of the humus layer, core samples of the underlying mineral soil, 25 cm^2 in cross-section area, were drilled to the depth of 9 cm. The cores were sliced in the field in three equal layers: 0–3 cm, 3–6 cm, and 6–9 cm, which were put into individual plastic bags.

The samples were sent immediately in an insulated box by road to the laboratory in Helsinki, a distance of 840 km. The time from sampling to the beginning of sample treatments did not exceed 48 h.

In the laboratory the humus and the mineral samples were treated in different ways. A pooled sample of humus was made as follows. Five separate weighings of 1 g were made from each of the five cakes of humus. These samples were transferred to individual 99-ml blanks of sterile water with glass beads. They were vigorously shaken by hand for 5 min after which the five suspensions of one cake were pooled in a larger flask. When the other four cakes of humus from the same sampling site had been treated similarly, 100-ml volumes of suspension from each large flask were pooled.

The suspension was serially diluted in 99-ml blanks of sterile water to give dilutions 10^{-2}, 10^{-4}, 10^{-6}, and 10^{-8}. One-millilitre and 0.1-ml aliquots were plated to give the 10-fold dilutions from 10^{-2} to 10^{-9}. The pour-plate method was used.

The mineral samples were pooled as follows. All five samples of the same depth of one sampling site were transferred *in toto* to a flask containing 200 ml of sterile water. The suspension was well shaken by hand for 5 min and decanted to an empty sterile flask. The coarse mineral residue in the first flask was rinsed three times with sterile water, and shaken each time as before. The washings were pooled with the suspension in the second flask. This suspension was filled to the 500-ml mark with sterile water, diluted, and plated out as described above.

Isolation and Maintenance of the Bacteria

Serial dilutions of the soil samples, handled and pooled as described above, were plated in a modification of the isolation medium (IM-medium) used by Taylor (1951).

The soil extract of the IM-medium was prepared as described by James (1958). For isolation media the soil extract was prepared from the humus layer of the soil sample in question, and for maintenance and testing media it was prepared from a mixture of humus from all the sampling sites. The pH of the medium was adjusted to 5.5, the agar content was raised to 20 g/litre, and 10 mg of actidion was included per litre for isolation purposes. Fungal growth on the isolation plates was further restricted by addition of 55 μg of mycostatin per millilitre to the 99-ml portions of sterile water used for dilution of the soil samples.

In a study on bacteria of a Scottish pine forest, Goodfellow *et al.* (1968) used stronger concentrations of the named fungistatics. We found the reduced amounts sufficient to allow isolation of bacteria from the northern soil material without problems with contaminating fungi.

After incubation for 10 days at room temperature (22–24 °C) all the colonies from plates containing less than 100 colonies, or from sectors of such plates, were picked as stab cultures into semisolid IM-medium (the modified IM-medium described above without fungistatics and with the agar content reduced to 3 g/litre). Extension of incubation from 10 to 14 days did not increase the colony counts of the plates. The stab cultures were incubated at room temperature for a week and kept at 4 °C between transfers. Each culture which grew in the semisolid IM-medium was examined for morphology and motility in water mounts under phase-contrast optics, and in Gram-stained smears (Skerman 1967, p. 279) under bright-field optics, respectively, of young (1st day of visible growth) and old (one additional week of incubation) IM-medium slant cultures. Isolates considered non-axenic were omitted, as were cultures of streptomycetes or yeasts. When necessary, purity was checked on IM-medium streak plates.

TABLE 1. Number of isolates studied from the different layers of the sampling sites

Layer	Sampling site (years passed since clear-cutting) 0 (intact forest)	4	7	13*a*	13*b*
Humus	48	49	50	49	45
Mineral, 0–3 cm	30	30	30	30	30
" 3–6 "	30	30	30	30	23
" 6–9 "	30	30	30	30	30
Total	138	139	140	139	128
Grand total 684					

The numbers of cultures remaining after reduction resulting from failure of growth, impurity, or indications of streptomycetes or yeasts are shown in Table 1. The investigation was confined to these 684 isolates which were kept as stab cultures in the semisolid IM-medium as described above with transfers every 6 months. The isolates were predominantly Gram-negative, nonpigmented, or pigmented rods. Only 4% of them were endospore-forming rods, 14% showed some degree of Gram variability, and less than 1% were purely Gram-positive. Compared with bacterial populations isolated from other northern 'tundra sites' (Rosswall and Clarholm 1974) and coniferous forests (Goodfellow *et al.* 1968; Sundman 1970), the bacterial populations of this investigation contained more Gram-negative, and less sporeforming isolates resembling in this respect the isolates from Hardangervidda in Norway (Rosswall and Clarholm 1974).

The Testing Procedure

As far as possible the physiological tests were performed with the aid of a 100-probe multipoint inoculator serving either (*a*) 12 × 100 mm test tubes in quadratic racks of 10 × 10 positions or (*b*) round, 18-mm-diameter compartments for plate inoculations in the same formation of 10 × 10, obtained with the aid of 'hole batteries,' composed of 100 pieces of stainless steel tube joined by welding, and placed in solidifying agar medium, poured into square racks made up of glass plates and low metal frames constructed to fit the 'hole batteries.' The instrument was locally constructed according to drawings obtained by one of us (SN) from Dr. Graham Skyring (at the time working at the Department of Agriculture, Ottawa, Canada).

Stab cultures in semisolid IM-medium not more than 7 days old were used as inocula. All cultures were incubated at room temperature if not otherwise stated.

The Tests

For each of the 684 isolates the results of 36 physiological tests were recorded. The tests are listed in Table 2 together with the percentages of positive results among all the isolates. The tests were performed as previously described (Sundman 1970) except for the following modifications and amendments. The results were recorded after 21 days (acid from sugars, degradation of cellulose, and chitin), after 14 days (reduction of methylene blue, and growth at 2 °C), or after 7 days of incubation (the other tests).

Degradation of cellulose was determined as clarification of IM-medium which contained 1% (w/v) of cellulose prepared from Whatman filter paper No. 4, according

TABLE 2. List of tests applied and percentages of positive results among the 684 isolates studied

Test	% positive results
1 Acid from glucose under vaspar seal	25.4
2 " " " aerobically	93.1
3 " " mannose "	94.9
4 " " arabinose "	94.3
5 " " xylose "	84.1
6 " " rhamnose "	53.5
7 " " maltose "	50.9
8 " " sucrose "	25.9
9 " " cellobiose "	54.8
10 Hydrolysis of cellulose	9.5
11 " " starch	11.8
12 " " chitin	4.8
13 " " gelatine	35.2
14 " " casein	30.3
15 Utilization of protocatechuic acid	82.0
16 " " vanillic acid	12.9
17 " " vanillin	13.0
18 " " lignosulfonate	3.2
19 Lipolysis of Tween 80	70.3
20 " " egg yolk	36.3
21 Lecitinase	33.3
22 Phosphatase	36.8
23 Dissolution of $CaHPO_4$	24.4
24 Urease	42.7
25 Acetamidase	87.1
26 Ammonification	91.4
27 Nitrate reductase	25.4
28 Sulphide produced from cysteine	11.1
29 Oxidase	23.2
30 Reduction of methylene blue	13.5
31 Sensitivity to penicillin (100 IU/ml)	11.5
32 Resistance to crystal violet (100 mg/litre)	88.6
33 " " basic fuchsin (1 g/litre)	93.4
34 Growth at 2 °C	78.2
35 " " 35 °C	14.5
36 Minimum pH, permitting growth, pH 3.5	6.1
4.0	38.7
4.5	32.7
5.0	14.6

to Berg *et al.* (1972), and which was stratified over plain IM-medium.

Degradation of chitin was likewise determined as clarification of IM-medium which contained 1% (w/v) of finely dispersed chitin and was stratified over IM-medium. The chitin was prepared from a commercial preparation (Fluka) which was soaked overnight in 2% (w/v) potassium hydroxide, washed with water, neutralized with diluted hydrochloric acid, and washed again with water. The neutral, washed suspension was homogenized by the aid of an Ultra Turrax homogenator applied for 15–20 min in 5-min periods.

For determination of hydrolysis of gelatin 0.2% (w/v) was included in IM-medium.

Hydrolysis of casein was noted as clarification of IM-medium which contained 1% (w/v) of skim milk powder.

Utilization of phenolics was determined on IM-medium which contained 0.02% (w/v) of protocatechuic acid, vanillic acid or vanillin, or 0.05% (w/v) of lignosulfonate (Peritan Na obtained from Norcem, Oslo, Norway) as described by Sundman and Näse (1971).

Ammonification was determined as alkalization of IM-medium modified to contain 5 g of tryptone, 3 g of yeast extract, 2 g of KH_2PO_4 but no other phosphates, and 0.02 g of phenol red per litre.

Production of sulphide from cysteine was registered with the lead acetate paper method (Skerman 1967, p. 239) in agar-less liquid IM-medium which contained 0.1 g of cysteine per litre.

Tolerance of dyes was determined according to recommendations by Fung and Miller (1973) using 100 mg of crystal violet and 1 g of basic fuchsin, respectively, per litre of IM-medium modified as for the ammonification test, but omitting the phenol red.

The test results were scored as plus or minus except for the minimum growth-permitting pH which was recorded as such.

Preliminary Editing of Data

At first all tests which had yielded less than 10% or more than 90% positive scores were discarded. The reason for such action was the intention of applying the X^2 test for comparing frequencies of test results between populations. As the total number of isolates from most samples was only 30, too low frequencies for the X^2 test would have followed from tests with the above degree of asymmetry.

By this rule, the test for acid production aerobically from glucose (93% positive), mannose (95%), and arabinose (94%), hydrolysis of cellulose (9%), utilization of lignosulfonate (3%), ammonification (91%), and tolerance of 0.1% (w/v) basic fuchsin (93%) were removed from the data. The test for hydrolysis of chitin, although positive only for 5% of the cultures, was retained against this rule because of its supposed association with invertebrate residues, the amount of which had been studied in the zoological part of the work.

Next, the correlation coefficients between pairs of tests were examined. Extremely high correlation was taken as a sign to consider removal of one of the pair for reasons of redundancy, but only if biochemical or other independent reasons also supported the statistical result. The test for utilization of vanilline was removed as redundant with the utilization of vanillic acid. Lecithinase test (egg yolk) correlated highly with the lipolysis test (egg yolk). The latter was retained as the more reliable of the two.

Mathematical Methods

Condensation of the remaining 28 tests into fewer dimensions was made by factor analysis. The decision to choose factor analysis from among the multivariate methods was partly based on previous successful application of the same (Sundman 1970) and partly on the random nature of the isolate collection (Seal 1964, Cattell 1965).

Maximal correlation coefficients associated with each test were used as the communality estimates. The principal axes solution for 10 factors was first calculated. Decision to limit the analysis to seven factors was reached after inspection of eigenvalues and factor loadings. The last accepted factor had an eigenvalue

0.412, and the sum of eigenvalues of the first seven factors was 102% of the sum of communality estimates of the 28 tests, indicating adequate condensation of all data. Rotation towards a simpler structure was made by the Varimax technique.

The factor scores, or projected values, of isolates on the seven axes (factors) were subsequently used in place of the 28 test results in comparisons of population.

Two-way analysis of variance was applied in testing the existence of significant effects both in respect of time since clear-cutting of sampling site and in respect of depth of sample stratum, or their interaction.

For comparing and illustrating the general similarity of two populations (A and B) in the multidimensional space defined by the seven factors, squared Euclidean distances between their centroids (D^2_{AB}) were computed:

$$D^2_{AB} = \sum (a_i - b_i)^2, \quad i = 1, 2, \ldots, 7$$

where a_i and b_i are the mean factor scores of the two populations on the ith factor.

Results

Evaluation of the Factors

The isolates of this study form 20 populations, which can be grouped in a 2-way table (Table 1) according to sample *depth* and to sampling sites, which were selected according to *years* passed since clear-cutting.

Two-way analysis of variance was carried out to test the differences between the mean factor scores of these populations. This was done separately for each factor. Save for factor number 2, the (depth) × (years) interaction was significant at 99.9% probability which makes simple generalizations difficult. Nevertheless, the next step was to inspect the influence of the main effects separately. The total population was grouped first according to sample depth and next according to sampling site (equivalent to years since clear-cutting). The positions of these populations in the factor space were determined for each factor.

The ability of the factors to differentiate between populations from different soil strata is illustrated in Fig. 1. Factors 3 and 5 display the widest range, with the distinction that factor 5 effects a rather clean separation of humus from mineral populations, whereas factor 3 concentrates more on the differentiation of mineral layers. Factors 1, 2, 4, and 7 seem much less effective.

A similar rough evaluation of factors can be made in the time dimension by averaging the factor scores of all depths of every site. The result is illustrated in Fig. 2.

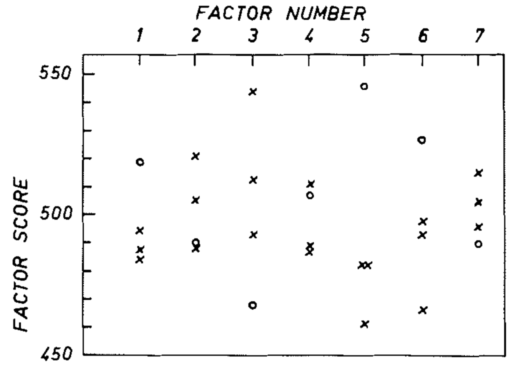

FIG. 1. Distribution of the average factor scores (arbitrary units) of humus and mineral populations. Values averaged over all years (sites). (×), Mineral populations; (○), humus populations.

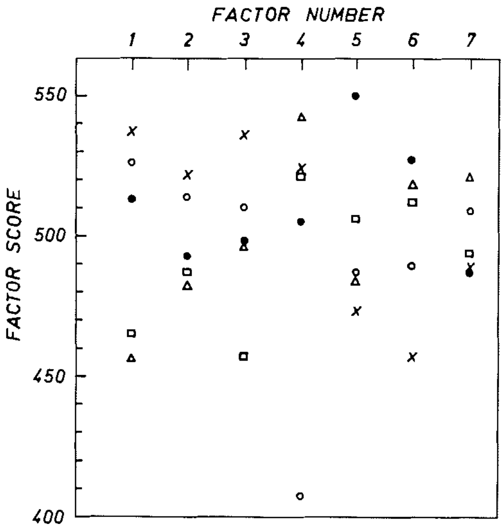

FIG. 2. Distribution of the average factor scores (arbitrary units) of populations from different sites (years). Values averaged over all layers. (●), No cutting; (○), 4 years after clear-cutting; (×), 7 years after clear-cutting; (△, □), 13 years after clear-cutting.

In marked contrast with the depth dimension (Fig. 1), factor 4 now shows by far the widest range of values. It is, therefore, the most effective factor at separating the sampling sites. The main function of factor 4 is, however, to separate the 4-year population from all the rest, indicating a special situation after 4 years. Factor 5 effects separation of the control sample from all others and may also be rather informative despite its smaller range.

In summary of Figs. 1 and 2 factor 4 seems to concentrate on the detection of changes after 4 years, and factor 5 seems the most promising general detector of population changes caused in different soil layers by the clear-cutting of timber. Factors 1, 3, and 6 are of intermediate value and factors 2 and 7 seem useless for such purposes.

To disclose the nature, direction, and extent of population changes implicated by the observed differences in average factor scores, two further approaches were tried: the Euclidean distances between population means and inspection of the original frequencies of test scores in the light of the most promising factors 4 and 5.

Squared Euclidean Distances

Because of many significant interactions in the analysis of variance, and because of some clear humus–mineral contrasts in Fig. 1, it was considered appropriate to deal separately with the populations isolated from the humus and the mineral layers. The squared Euclidean distances between point centroids of populations from different sites are presented for the pooled populations of the mineral layers in Table 3 and for the humus layer in Table 4.

Lacking a theoretical measure of stochastic variability, the significance of squared Euclidean distances between populations was estimated using the squared distance between the two populations of the same age (13*a* and 13*b*) as a rough measure of random variation. Thus a squared distance of roughly 80 units was considered well within random variation and only values at least twice that figure were assumed to indicate significant dissimilarity.

TABLE 3. Squared Euclidean distances (arbitrary units) between population point centroids in seven-dimensional factor space. Pooled mineral populations of different sites (0, 4, 7, and 13 years after clear-cutting)

	0	4	7	13*a*	13*b*
0	0				
4	354	0			
7	342	279	0		
13*a*	213	380	277	0	
13*b*	144	486	286	81	0

TABLE 4. Squared Euclidean distances (arbitrary units) between population point centroids in seven-dimensional factor space. Humus populations of different sites (0, 4, 7, and 13 years after clear-cutting)

	0	4	7	13*a*	13*b*
0	0				
4	195	0			
7	210	217	0		
13*a*	179	218	325	0	
13*b*	164	142	348	66	0

The first column of figures in both tables gives the distances from control. It seems that the mineral populations (Table 3) after 4 and 7 years are quite different from the control, and that distinct signs of nearing the control appear after 13 years of development. The same is less evident in the humus populations (Table 4).

The squared distances between humus and mineral populations of each site were also calculated and are shown graphically in Fig. 3. In undisturbed soil (0 years) the humus and mineral populations were no more distant (77 units) than is accountable by random variation, but 4 and 7 years after clear-cutting the populations in the two soil layers are quite significantly different. After 13 years, the humus and mineral populations no longer differ much more than can be expected by chance.

Interpretation of Population Differences

Squared Euclidean distances give no clues about the nature or direction of population changes. After significant differences had been found both in respect of years and of depth, with factors 4 and 5 most clearly accounting for them, the loadings of these factors were examined. (Factor loadings indicate the relative weights with which each test has participated in determining the orientation of the factors.) A brief summary is presented in Table 5.

Orientation of factor 4 thus seems to have been mainly determined by two characters, dis-

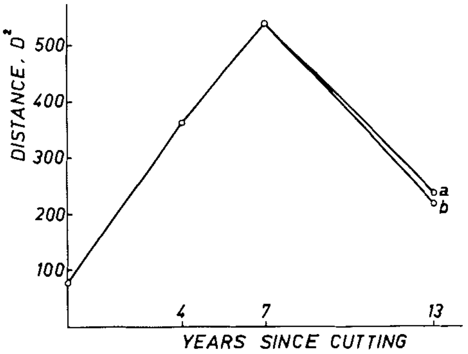

FIG. 3. Squared distances between point centroids of humus and mineral populations in seven-dimensional factor space, presented as function of time after clear-cutting.

TABLE 5. The five most prominent tests in factors 4 and 5 arranged in decreasing order of importance according to the absolute value of their factor loadings

Factor 4		Factor 5	
Test	Loading	Test	Loading
$CaHPO_4$ dissolution	0.516	Lipolysis (egg yolk)	0.523
Acid from sucrose	0.506	Casein hydrolysis	0.520
Urease	0.269	Minimum growth-permitting pH	0.435
Lipolysis (egg yolk)	0.204	Acid from rhamnose	−0.338
Nitrate reduction	−0.196	Reduction of methylene blue	0.333

solution of $CaHPO_4$ and acid production from sucrose. Relatively high correlation of the two tests ($r = 0.343$; df 682) indicated that the characters were contained in the same cultures to a significant degree. The orientation of factor 5 was determined somewhat more uniformly by several tests.

How the characters actually 'behaved' in the study can be seen from the changes in relative frequencies of positive cultures with time. Figure 4 illustrates this for four characters.

Two distinct types of temporal development appear to be common. One is a long-lasting change (positive or negative) extending through 4 and 7 years (curve H in Fig. 4B and curves M in Fig. 4C and D), and the other is a short-lived disturbance, apparent only in the 4-year samples (curves M in Fig. 4A and B and curves H in Fig. 4C and D). These two classes of temporal trend found their expression in factors 4 (Fig. 2) and 5 (Fig. 1) and in the squared Euclidean distances in seven-dimensional space (Fig. 3). As is seen from Fig. 4, each type of temporal trend can be found with the bacterial populations of both humus and mineral layers. This explains the significant (depth) × (years) interactions detected by the analysis of variance.

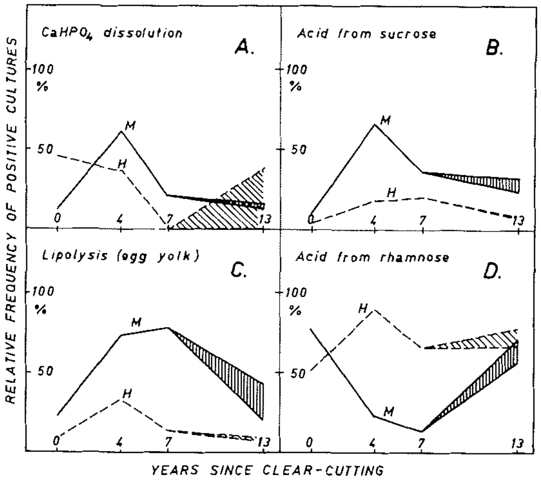

FIG. 4. Relative frequencies of positive cultures (in % of total cultures examined) arranged according to increasing age from clear-cutting. The hatched areas indicate the extent of variation between the two 13-year sampling locations. H = humus data, M = pooled data for mineral layers.

Discussion

Clear-cutting of timber causes long-lasting changes in the forest environment. Removal of the tree canopy effects both soil moisture and temperature. Decomposable organic matter is suddenly added in large quantities to the soil surface, but the usual continuous shower of needles and twigs ceases. Activity of tree roots stops. It is to be expected that such changes also influence the bacterial populations in the soil.

An attempt has been made to resolve and describe changes of the characteristics of the soil-bacterial populations which might have been caused by clear-cutting. The main function of the method applied, factor analysis and calculation of the squared Euclidean distances between population centroids, was to condense the results of numerous biochemical characters of several hundred bacterial isolates into fewer figures, and to draw out the population features which were the most profoundly affected. This approach has been successfully used earlier for the description of differences between field and forest soil-bacterial populations (Sundman 1970).

It was concluded that clear-cutting had caused a significant relative increase in caseolytic and lipolytic, rhamnose-negative organisms which also had a low acid tolerance. The change was rather permanent, lasting at least 7 years, and concerned particularly the populations of the mineral soil layers. Independent of this change, a short-lived increase of acid producers from sucrose, also capable of $CaHPO_4$ dissolution, took place in the populations of the mineral layers. A temporary change of the same general type but of smaller magnitude was apparent in the humus layer, too. The organisms concerned were lipolytic but rhamnose-positive.

The fact that the temporal and spatial differences seemed to be most clearly expressed in factors 4 and 5 indicated that quite a large part of the structure of the bacterial populations was unaffected by clear-cutting. This unaffected biological structure could have been described by studying the tests prominently represented in factors 1 to 3 but this has been considered irrelevant to the topic.

It seemed that bacterial populations of the humus and of the mineral layers were not very different in the undisturbed sampling site (control). Because of clear-cutting the populations of the two layers appeared to drift apart, with the difference culminating somewhere near 7 years after clear-cutting. The populations in the mineral layer seemed to be more profoundly disturbed than that of the humus layer. Evidently, the cessation of tree root activity was a more important influence on the soil bacterial flora than the addition of litter to the surface. After 13 years, signs of return to the original state were evident. In some features the cycle was shorter, with signs of disturbance only evident in the 4-year samples.

The same general trend prevailed in the invertebrate population studied by Huhta (1976) in monthly samples during two consecutive growing seasons.

If bacterial populations found in normal coniferous forest soils contribute to successful establishment of seedlings, then it may be concluded from this study that at least 10, possibly many more, years of recovery after clear-cutting might, under severe conditions, be necessary for successful reforestation. (Despite the same trend of divergence in soil population during some 10 years after clear-cutting, as was found for the soil invertebrates also in southern Finland, clear-cutting did not prevent rapid reforestation there.)

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